



The Evolution of Pathogen Discovery in Liberia

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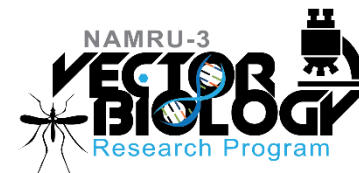
Research Associate

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Website: LIBResearch.org



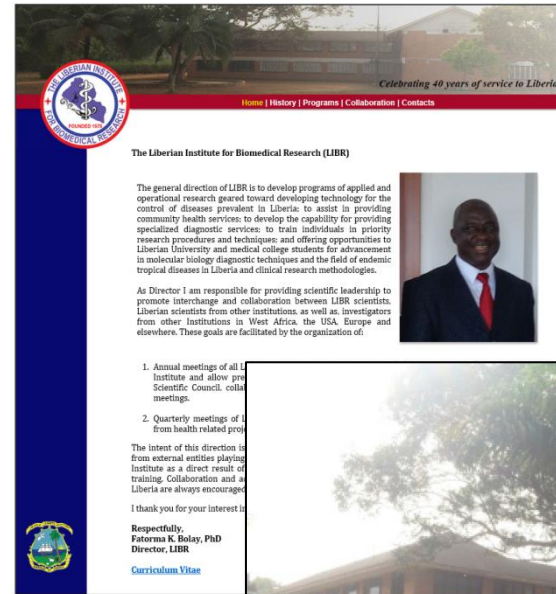
Biodefense solutions to protect our nation





Liberia Institute for Biomedical Research

- Director: Fatorma K. Bolay, PhD.
- In operation since 1975.
- Has been the site of international collaborations studying endemic diseases including but not limited to:
 - Schistosomiasis
 - Malaria
 - Ebola virus
 - Hepatitis
 - Onchocerciasis
 - HIV
 - Lassa Fever
- Houses the diagnostic capability of the Liberian National Reference Lab
- Website: <http://libresearch.org>





Liberia Institute for Biomedical Research

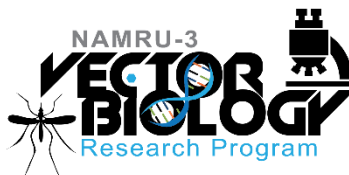
- LIBR is operated by the Board of Governors, responsible for all policies affecting the Institute and its activities.
- The Liberian Institute for Biomedical Research (LIBR) was founded 40 years ago and at one time was considered the scientific hub of West Africa.
- Due to a long and brutal fourteen-year war, LIBR was left a shell of its former self with close to no funding or resources.





Armed Forces Health Surveillance Center

- March 2010: **NAMRU-3** was requested by AFRICOM to investigate the high incidence of malaria among U.S. Forces
- History of Malaria Cases
 - 2003: U.S. Marines 80 / 225
 - 2010: 16 cases, 1 death in DEC 2009
 - 90% *Plasmodium falciparum*, 10% *P. ovale*
 - 2010 MID-year GEIS money used to support spray operation and mosquito workshop
- 2011 and 2012 GEIS funded projects





Distribution of *Anopheles* in Liberia

Objectives:

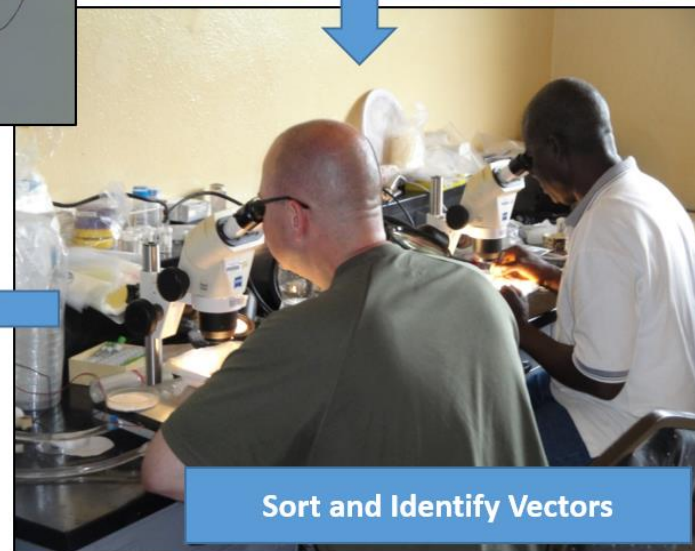
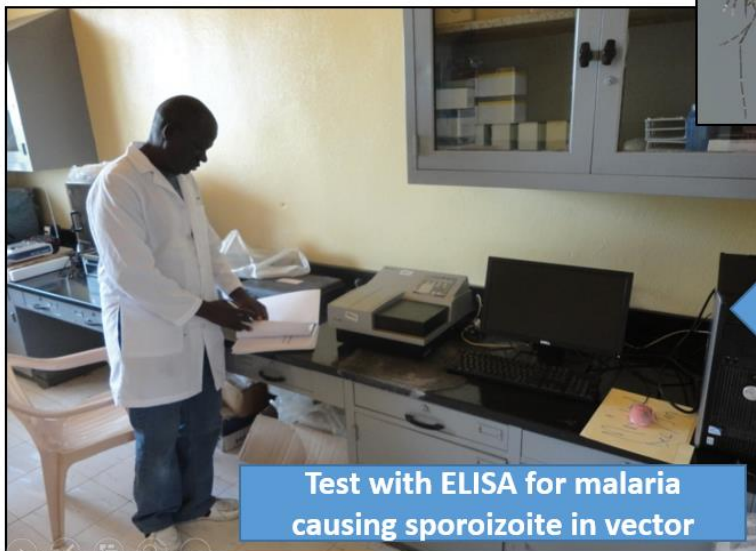
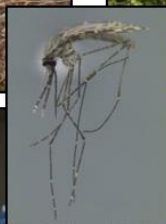
- Implement a country-wide mosquito surveillance.
- Map *Anopheles* spp. distribution using GIS
- Support PMI initiatives





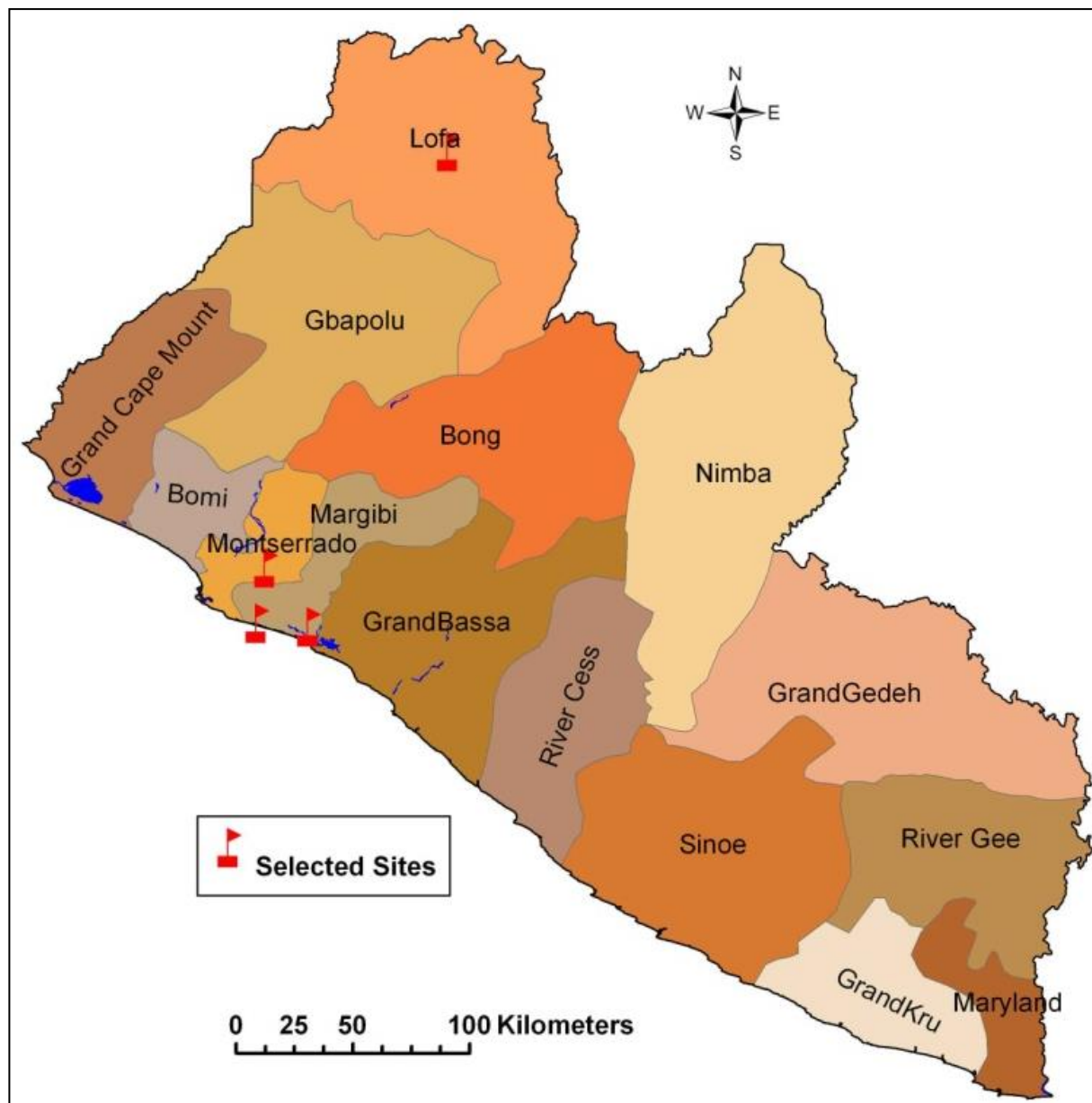
Distribution of Anopheles in Liberia

Surveillance and Detection





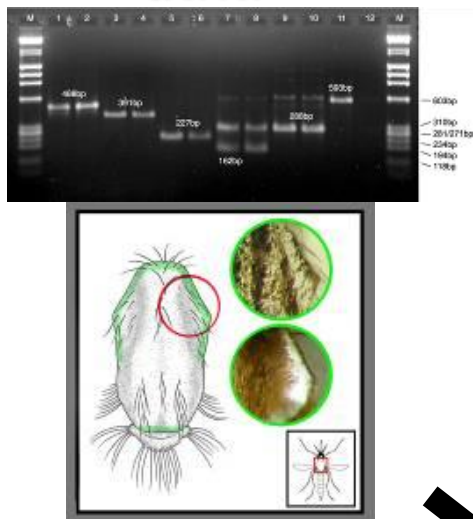
Distribution of Anopheles in Liberia



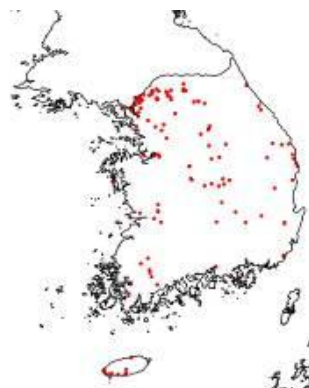


Distribution of Anopheles in Liberia

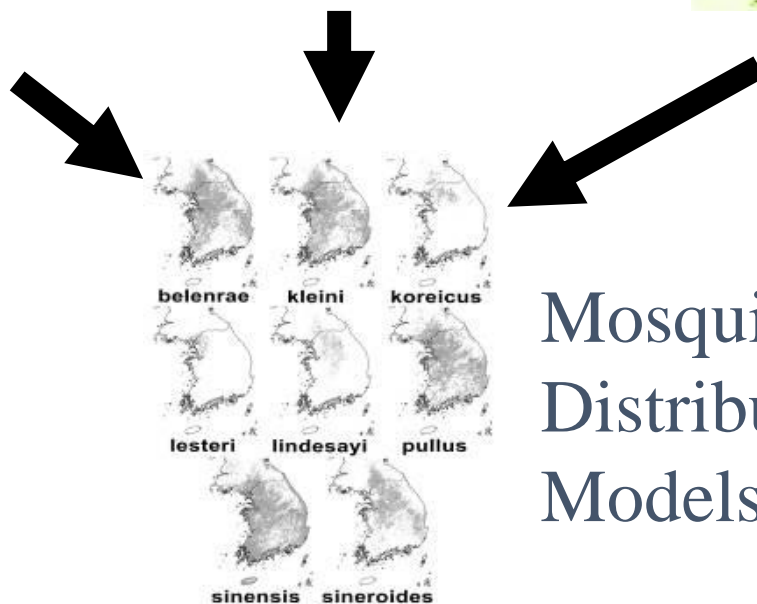
Taxonomic
data



Point occurrence
data



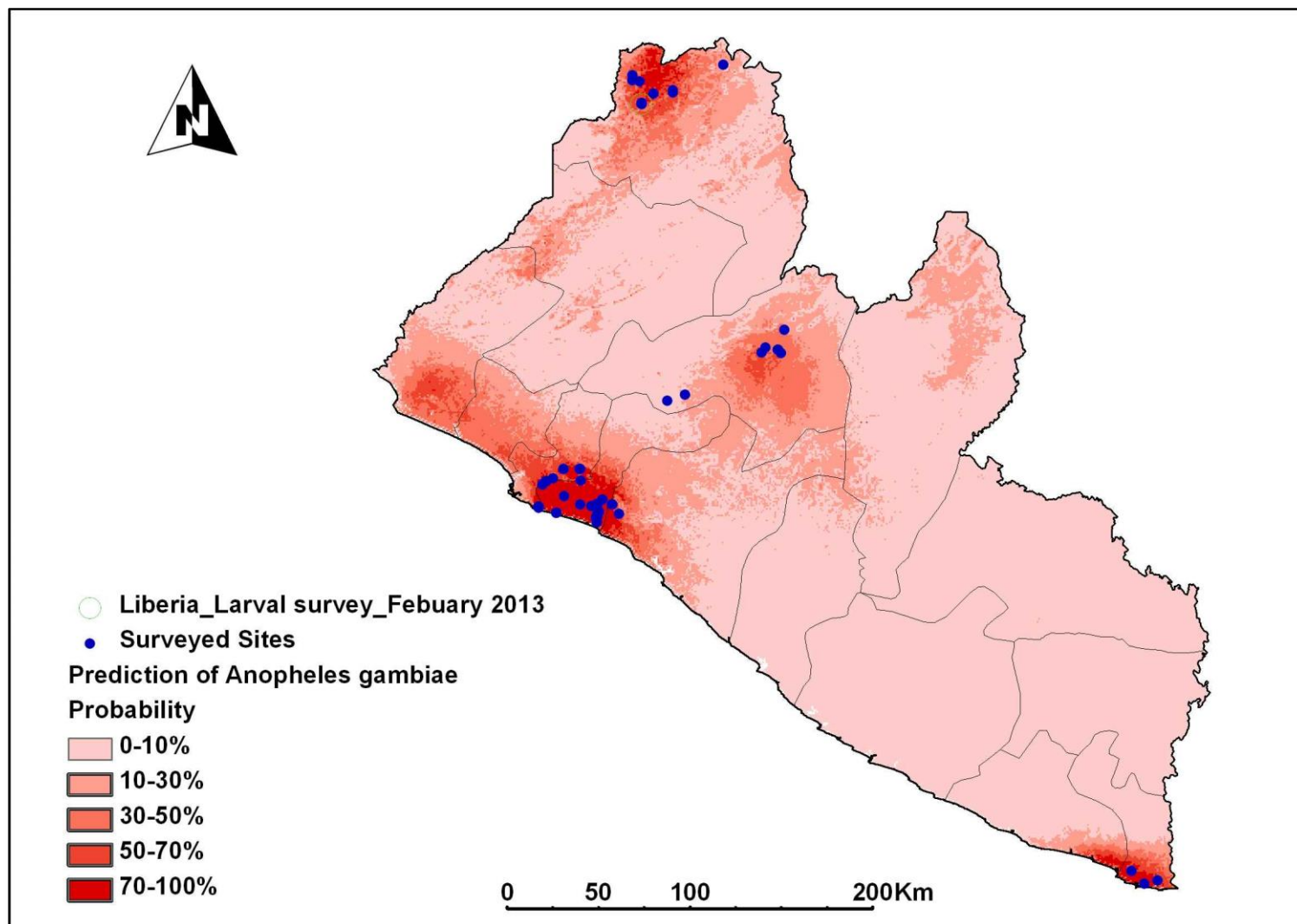
Environmental
data



Mosquito
Distribution
Models



Distribution of Anopheles in Liberia





Surveillance of Arboviruses Potentially Involved in Human Febrile Illness in Liberia.

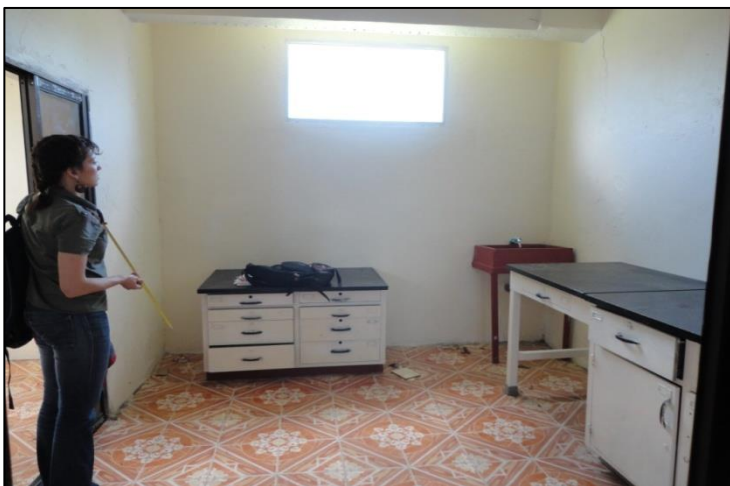
Capability Building





Surveillance of Arboviruses Potentially Involved in Human Febrile Illness in Liberia.

Capability Building



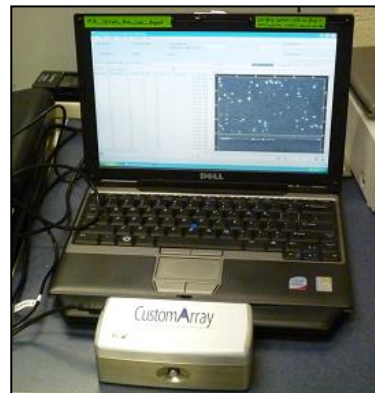


Surveillance of Arboviruses Potentially Involved in Human Febrile Illness in Liberia.

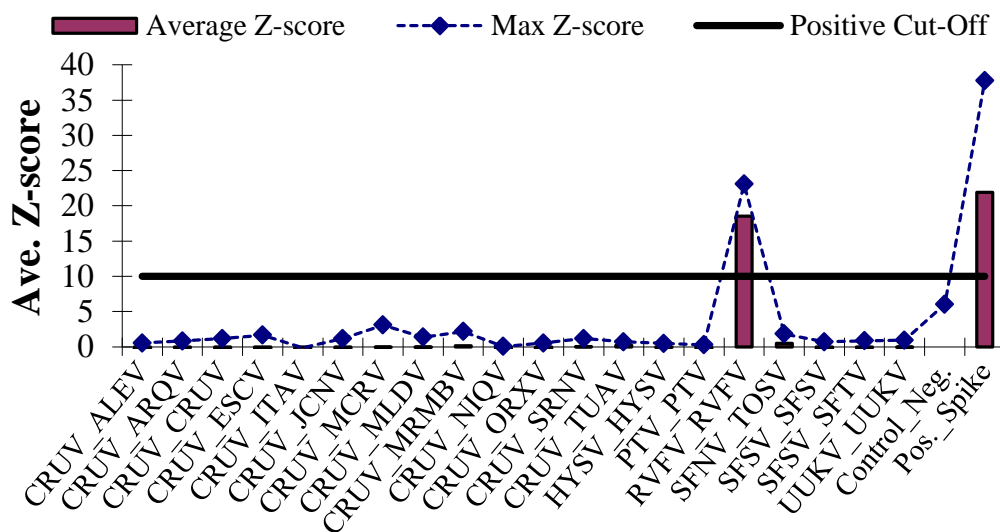




Surveillance of Arboviruses Potentially Involved in Human Febrile Illness in Liberia.



No.	Mosquito species (n)	PCR result	S seg.	M seg.	L seg.	S seg.	M seg.	L seg.
S-8	Unidentified sandfly (1)	Faint ~370 bp	Neg.	Pos.	Neg.	Neg.	RVFV^g	Neg.
S-32	Unidentified sandfly (1)	Faint ~370 bp	Neg.	Pos.	Neg.	Neg.	RVFV	Neg.

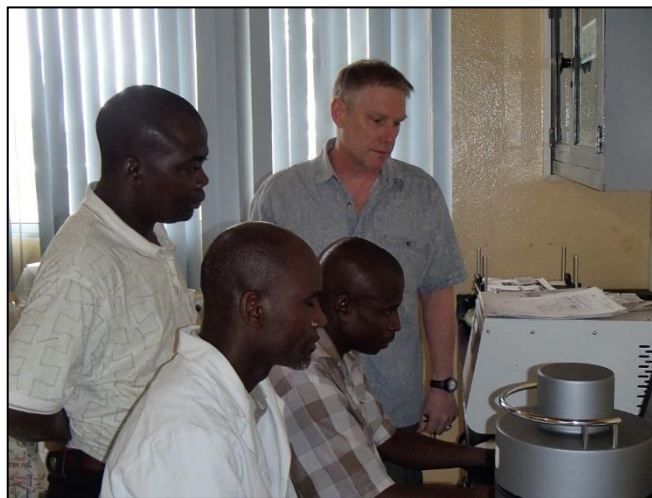


Phlebovirus M segment





Ebola Outbreak Diagnostics.





Ebola Outbreak Diagnostics.



Previous NAMRU-3 laboratory and human capacity building utilized during Ebola outbreak





From conventional PCR to High-throughput sequencing.





From conventional PCR to High-throughput sequencing.

Monitoring of Ebola virus: Liberian counties surveilled.

- Samples derived from EVD patients of seven coastal counties.
- **~1700 positive samples in collection spanning June 2014 to Present.**
- ~400 samples met criteria for sequencing by SISPA amplification (<25 Ct).
- 25 nearly complete genomes were published in the initial report in EID in Mar 2015.






From conventional PCR to High-throughput sequencing.

Results from the LIBR Sequencing Center: Ebola virus monitoring.

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Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia
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Volume 21, Number 7—July 2015
Research
Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia
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[Suggested citation for this article](#)

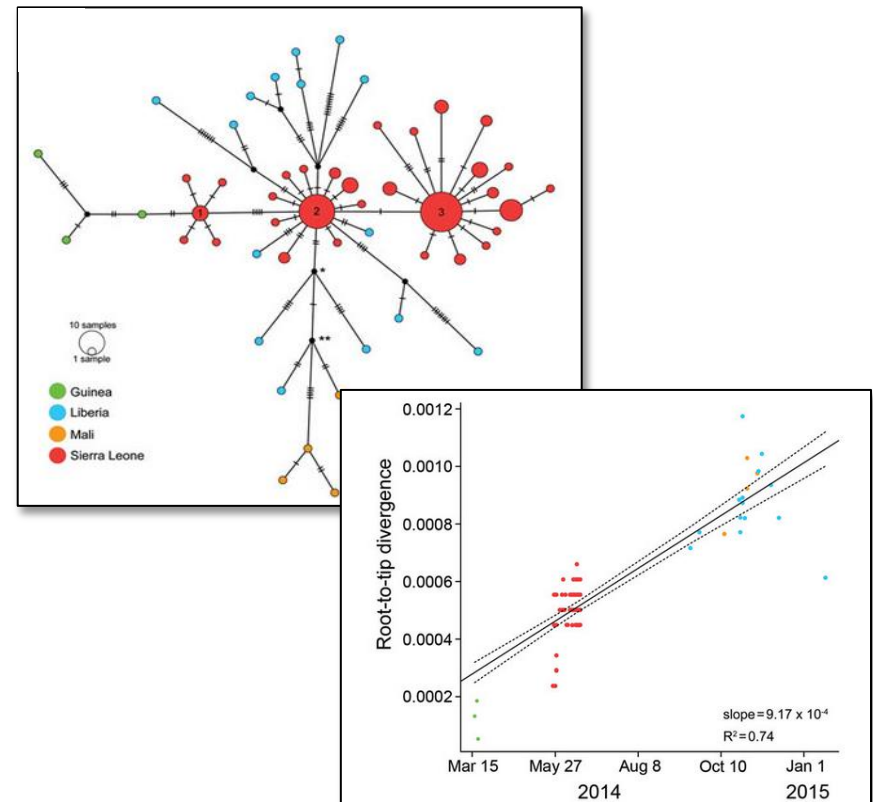
On This Page
[Materials and Methods](#)
[Results](#)
[Discussion](#)
[Suggested Citation](#)
Figures
[Figure 1](#)
[Figure 2](#)



From conventional PCR to High-throughput sequencing.

Monitoring of Ebola virus: Viral Evolution

- Introduction events – Liberian outbreak most likely occurred from a single introduction of a clade 2 virus.
- Mutation rates agree with those published by other groups: $\sim 9.17 \times 10^{-4}$
- Possible introduction from Liberia into Mali.





From conventional PCR to High-throughput sequencing.

Monitoring of Ebola virus: Target Erosion

- Minimal target erosion observed in Diagnostics used at LIBR.
- More substantial changes seen in publicly available Zaire Ebola virus probes but not all are in use.
- Minimal target erosion seen in therapeutics since the original report in *mBio*, 2015.
- Risk assessment for efficacy: Low.**





From conventional PCR to High-throughput sequencing.

Monitoring of Ebola virus : Complete, timely, sequence characterization

Sample ID	Patient age, y/sex	County of residence	Test date	Sample type	Average C _t value†
LIBR0993	33/M	Montesserrado	2015 Jan 20	Plasma	19.5
LIBR1195	35/M	Margibi	2015 Feb 2	Oral swab	22.5
LIBR1413	56 M	Montesserrado	2015 Feb 14	Plasma	22.5

- **Sample 1413 was the first sample completely prepared and sequenced by Liberian staff.**
- **Reports to the MOH, CDC, WHO and DOD were made available within 7 days of sample receipt for the February samples.**
- **The public report was made publicly available Apr 22, 2015.**



From conventional PCR to High-throughput sequencing.

Results from the LIBR Sequencing Center: Molecular Evidence of Transmission






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A-Z Index **A B C D E F G H I J K L M N O P Q R S T U V W X Y Z #**

Morbidity and Mortality Weekly Report (MMWR)

[MMWR](#)

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Possible Sexual Transmission of Ebola Virus – Liberia, 2015

Early Release
May 1, 2015 / 64(Early Release);1-3

Athalia Christie, MIA¹, Gloria J. Davies-Wayne, MPH², Thierry Cordier-Lasalle, DESS², David J. Blackley, DrPH¹, A. Scott Laney, PhD¹, Desmond E. Williams, MD, PhD¹, Shivam A. Shinde, MBBS², Moses Badio, MSc³, Terrence Lo, DrPH¹, Suzanne E. Mate, PhD⁴, Jason T. Ladner, PhD⁴, Michael R. Wiley, PhD⁴, Jeffrey R. Kugelman, PhD⁴, Gustavo Palacios, PhD⁴, Michael R. Holbrook, PhD⁵, Krisztina B. Janosko, MS⁵, Emmie de Wit, PhD⁵, Neeltje van Doremalen, PhD⁵, Vincent J. Munster, PhD⁵, James Pettitt, MS⁵, Randal J. Schoepp, PhD⁴, Leen Verhenne, MD⁶, Iro Evlampidou, MD⁶, Karsor K Kolie, MPH³, Sonpon B. Sieh³, Alex Gasasira, MBChB², Fatorma Bolay, PhD⁷, Francis N. Kateh, MD³, Tolbert G. Nyenswah, MPH³, Kevin M. De Cock, MD¹

On March 20, 2015, 30 days after the most recent confirmed Ebola Virus Disease (Ebola) patient in Liberia was isolated, Ebola was laboratory confirmed in a woman in Monrovia. The investigation identified only one epidemiologic link to Ebola: unprotected vaginal intercourse with a survivor. Published reports from previous outbreaks have demonstrated Ebola survivors can continue to harbor virus in immunologically privileged sites for a period of time after convalescence. Ebola virus has been isolated from semen as long as 82 days after symptom onset and viral RNA has been detected in semen up to 101 days after symptom onset (1). One instance of possible sexual transmission of Ebola has been reported, although the accompanying evidence was inconclusive (2). In addition, possible sexual transmission of Marburg virus, a filovirus related to Ebola, was documented in 1968 (3). This report describes the investigation by the Government of Liberia and international response partners of the source of Liberia's latest Ebola case and discusses the public health implications of possible sexual transmission of Ebola virus. Based on information gathered in this investigation, CDC now recommends that contact with semen from male Ebola survivors be avoided until



From conventional PCR to High-throughput sequencing.

Transmission Molecular Evidence : Possible Sexual Transmission

TABLE. Course of Ebola in survivor A and family members — Liberia, 2014					
Relationship to survivor A	Age (yrs)	Date of symptom onset	RT-PCR results	Test dates	Date of death
Brother	62	August 22	Positive	September 5	Unknown (before September 5)
Brother	36	September 9	Not done	—	September 25
Survivor A	46	September 9	Indeterminate	September 28	Living
			Negative	October 3	
Daughter	14	September 16	Not done	—	September 23–28
Son	12	October 2	Positive	October 11	Unknown
Abbreviation: RT-PCR = reverse transcription–polymerase chain reaction.					

Christie et al, *MWWR*, 2015.

- Patient 1, contracted and died of Ebola virus 28 days after the last known positive case in Liberia in March of 2015.
- Case history of the known contacts of Survivor A.
- The viral genome did not match any of the most recent sequences.
- The only known contact determined from the epidemiological study was unprotected sexual intercourse with Survivor A. Declared convalesced (Ebola negative) in September of 2014.



From conventional PCR to High-throughput sequencing.

Transmission Molecular Evidence : SNP Evidence

Case	Relationship	4107	8592	16636	4384	12996	18405	6056	16514
MH13203	Patient 1	A	T	A	C	A	A	C	A
MH2012684	Survivor A	N	T	A	N	N	N	N	N
CDC659=0 CN1	Surv. Brother	G	N	G	C	N	N	C	A
CDC/NIH-1469	Surv. Ex Wife	G	A	G	N	C	N	A	G
LIBR0993	SPB (Jan 20, 2015)	G	A	G	A	C	-	A	G
LIBR1195	SPB (Feb 2, 2015)	G	A	G	A	C	-	A	G
LIBR1413	SPB (Feb 14, 2015)	G	A	G	A	C	-	A	G

- SPB Cluster follows a single introduction event and was the last circulating lineage known in Liberia.
- Low titers made sequence recovery difficult, however, signature recovered matches Survivor A.
- Other family members sequenced match the SPB cluster.
- **Resulted in a revision of the convalescent sexual contact recommendations by CDC and WHO.**



We would like to thank our collaborators!

- Naval Medical Research Unit No. 3 (NAMRU-3)
- United States Army Medical Research Institute for Infectious Diseases (USAMRIID)
- Armed Forces of Liberia
- National Reference Laboratory
- Liberian Ministry of Health; National Diagnostic Unit (NDU)
- National Malaria Control Program (NMCP)
- Center for Disease Control and Prevention (CDC)
- U.S. Agency for International Development (USAID)
- Navy Entomology Center of Excellence (NECE)